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EFFECT OF DIFFERENT IRRADIATION DOSES ON CHEMICAL CONSTITUENTS OF THE TWO SAMPLES OF EDIBLE MUSHROOM

Turk*, S.A.; El-Shafei, M.A; Ramadan*, M.A; M.E; Ahmed gad, M.R and Abd El-Hakeem**, H.I.**

**Agricultural Chemistry Department, Faculty of Agriculture, El- Minia university, El- Minia,. Egypt. **Food Technology Department, Institute Agric. Research Center, Giza Egypt.*

ABSTRACT

The goal of this study was to investigate the effect of different irradiation doses (2.5, 7.5 and 10 KGy) on crude proteins and total soluble sugars, L-ascorbic acid, total Phenolic compounds, total flavonoides, total carotenoids and some enzymes of two samples of mushroom *(Agaricus Bisporus)* and *(Peurotus Ostreatus).*

INTRODUCTION

Human relationships with mushrooms are ancient and fascinating. The Egyptian believed that they were agift from the god Osiris.

Mushrooms have long been used as a food or food flavouring material due do their unique and suitable, the typical flavour of mushrooms consists of non-volatile components Litch Field , (1967).

Ishikawa, et al., (1984) reported that mushroom contain various polyphenolic compounds recognized as an excellent antioxidant.

Mushroom can converst waste materials into human food these material are resistant to degreaddation because they contain mainly cellulose, hemi cellulose and lignin. The mycelium of mushrooms extracts extensive enzyme complexes which can directly attack and degrade these components and use it for their growth, *karma and Zadrazil. , (1988).*

Caglarrmak, N. (2007) reported that in Pleurotus Sajor- Caju has the highest vitamin content, and there was a variation in Vitamin C contents in the literature *koorapati et al., (2004)* reported that irradiation at doses of 1 and 2 KGy lowered (PPO) activity in the first

24h, either because of a modification of active site or change in conformation of the enzyme, and 1 KGy had more whiteness and firnmmes also the dose 5.2 KGy sample revealed more whiteness and much softer.

A self–eontained, prefabricated cabint laaded with ^{137}Cs to provide and additional processing option is being developed Electron beam facilities, widely used to irradiate medical equipment, have been built for food treatment. Energy penetration is about 1/5 inches in food products.

Beaulieu et al., (1992) reported that irradiated mushrooms undergo less browning compared to non-irradiated Samples during a prolonged storage.

Gautun et al., (1998) found that irradiated samples of mushrooms exhibited lower polyphenoloxidase activity compared to non- irratiated controls, and the low activity of polyphenoloxidase in irradiated mushroom may therefore be responsible for less browning in irradiated mushrooms cornpared to non-irratiated once.

Joan- Hwa et al., (2001) found that arabitol was found in bighest amount only in winter mushrooms (187 and 1.90 mg /g dry wt). in addition, glucose, mannitol and trehalose were found in vaired amounts in winter mushroom.

Caglarimak, N. (2007) reported that in Pleurotus Sajor- Caju has the higlest vitamin content, content, and there was a variation in Vitamin C contents in the literature.

The main objective of the present study was to investigated the effect of different irradiation doses on chemical constitutents of two samples of mushroom *(Agrrieus Bisporus)* and *(Pleuratus Ostreatus)*, were studied on crude proteim, and total soluble sugars, L- ascorbic acid, total phenolic compounds, total flavonoides, total conotenoids and some enzymes.

MATERIALS AND METHODS

Samples:

Two samples of mushroom button mushroom *(Agaricus Bisporus)* and oyster mushroom *(Pleurotus Ostreatus)* were obtained from Research Institute of Food Technology, Agricultaral. Research Center, Giza, Egypt. The shelf life of each *(Agaricus Bisporus and Pleurotus Ostreatus)* mushrooms were preserved using irradiation procedures.

Irradiated of mushrooms

One kilogram of fresh button mushroom *(Agarieus Bisporus.L.)* and the same weight from oyster mushroom *(Pleurotus Ostreatus)* were divided into four portions each of them, weighted 250 g and irratiated radiocesium C^{137} gamma cell unit 0.8478 rad/sec at different doses according to *(De Guzman and Caballing,. 2004)*, at Laboratories of Atomic Energy Authority, Nasr City, Egypt. As follows:

Control and irradiation dose (2.5, 7.5 and (0.0 KGy). After irradiation, mushroom samples were placed in PVC container and sheeting with polyethylene and stored at 4°C unit analyzed Determination of total nitrogen and crude protein (CP).

The kjeldahl procedure was used to determine the total nitrogen content in mushroom. This was per formed by Rapid nitrogen apparatus Model Buch 426, FRG. The crude protein was then calculated by multiplying nitrogen content by 4.38 as a factor for fruit bodies *(Crisan and Sands, 1978)*.

Extraction of Soluble Sugars:

Soluble sugars were extracted according to *(Macrae and Zand-Mghdlam 1978)*.

Determination of total Soluble Sugars (Tss):

Total soluble sugars were determined by the phenol- sulfuric acid method described by *(Dubois et al., 1956)* An aliquot (1ml) containing

10-70 μug carbohydrate reacted with phenol (1ml, 5% phenol in water) and concentrated H_2SO_4 acid (5ml), after 30 min the colour development was measured spectrophotometrically at 490nm.

The concentration of sugars in the unknown was determined by using Calibration curve constructed by solution containing known concentration (10 to 50 ug/mg of glucose).

Determination of ascorbic acid:

The indophenol method (2,6 di chlorophenol indophenol, 50 mg in 250 ml H_2O), as described by Mondy and Ponnamplam (1986), was used for determination of L-ascorbic acid concentration in mushroom.

Extraction and determination of Total Phenolic Compounds (TPCs):

TPCs was extracted from defatted sample (0.5g) by refluxing with 30 ml of methanol containin 1% HCL for 10 min, the extract was

centrifuged at 10.000 r.p.m for 10 min. the concentration of total Phenolic compounds in the methanolic extracts expressed as gallic acid equivalents. According to the methods of *Singleton and Rossi (1965)*.

Extraction and determination of total flavonoides (TFs):

Fresh, canned and irradiated powdered air- dried or oven – dried sample of mushroom extracts (5.Og) were extracted in a soxhlet extractor with 100 ml distilled water or ethanol for 1 hour and the extract filtered A A known volume of extract was placed in 10 ml volumetric flask, 0.3 ml 5% of $NaNO_2$ and 3ml (10%) of $AlCL_3$ were added 5 min later.

After 6 min, 2ml of 1 mole / liter^{-1} NaOH was added to the total and made up to 10ml with distilled.

The solution was mixed well and the intensity of pink color was measured at 510 nm using a spectrophotometer *(Taizhou Raio Factory) (Zhuang et al., 1992)*. All extracts were analysed in triplicate.

Extraction and Determination of total carotenoids:

Carotenoids were extracted from fruiting bodies of mushroom as described by *Megahed (1985).* The total carotenoids were calculated using *Heinonen and Marina (1989)* methods.

Determination of polyphenoloxid-ase activity (PPO):

PPO activity was assayed with Catechol as a substrate by a spectrophotometric procedure *(Jiang, 1999)*. The assay was performed using 0.5 ml 0.01 M Catechol, 5.0 ml of 0.1 M Sodium phosphate buffer (pH 7.0) and 0.5 ml of crude enzyme. The increase in absorbunce at 420 nm was recorded for 5 min. One unit emzyme activity was defined as the amount of the enzyme which caused a change of 0.001 in absorbance per minute**.**

Determinateion of tyrosinase activity:

Tyrosinase activities were assayed at 25°C in 0.1 M sodium phosphate buffer (pH 6.0) using 1mM tyrosine as substrate *(Green et al., 1975).*

Determination of Peroxidase (POD):

Peroxidase was extracted from 5g fresh weight of mushroom tissue ground in 50 ml of 50 mM phosphate buffer pH 7.0 and them centrifuged at 10.000 r.p.m for 10 min. The supernatant was used to determine POD activity, for the measurement of Guaiacol- dependent

peroxide activity, the reaction mixture contained 5mM Guaiacol, 10mM H_2O_2 and enzyme activity was determined by the increase in absorbance at 4-70 nm due to Guaiacol Oxidation *(Na kano and Asada 1981)*.

Determination of phenyl alanine ammounia Lyase activity (PAL):

PAL activity was measured using a modified method of *(Green et al., 1975)*. The reaction mixture consisted of 1.5 ml L-phenylalanine *(2mMin borate buffer pH8.8)* and 0.5 ml mushroom crude extract. The reaction mixture in was incubated at 40°C for 2h. The activity was expressed as increase in absorbance at 290 nm mg^{-1} protein min^{-1}.

Extraction and determination of proteases activity:

The total residual proteases activities (TRPA) of treated mushroom mushroom samples were assayed according to the method described by *(Basha and Beevers, 1975 and Salmia et al., 1978).*

RESULTS AND DISCUSSION

The role of irradiation on chemical constituents of mushroom:

Treating foods with ionizing enengy offers many benefits to consumers, retailers, and food manufactures. The benefits depend on the treatment used. Certainly the most important benefit is improved microbiological quality of food Addition benefits include the replacement of chemical treatment and extended shelf life, extended from 25-30 days.

Since irraditation dose not substantially raise the temperature of food being processed, nutrient losses are small and aften substantially less than other methods of preservation proteins, fats and carbohydrate are notably altered by irradiation *(Swallow 1991 and IAEA 1999)*.

Effect of different irradiation doses on some constituents of mushroom:

The effect of different dose of irradiation (2.5 , 7.5 and 10 KGy) on crude protein, total soluble sugars, L-ascorbic acid , total phemalic compounds, total flavonoides, total carotenoids and some enzymes activities in Figs (1-6) . The data in this work were very close to those reported by other investigators *(IAEA. 1996; IAEA. 1999; Sandhu)* and (*Poonam- Aggarwal 2001 Youssef et al., 2004)*, except for L- ascorbic acid which showed an increase in its amount especially the treatment with 2.5 KGy than others 7.5 and 10 KGy as cleared in Fig (3).

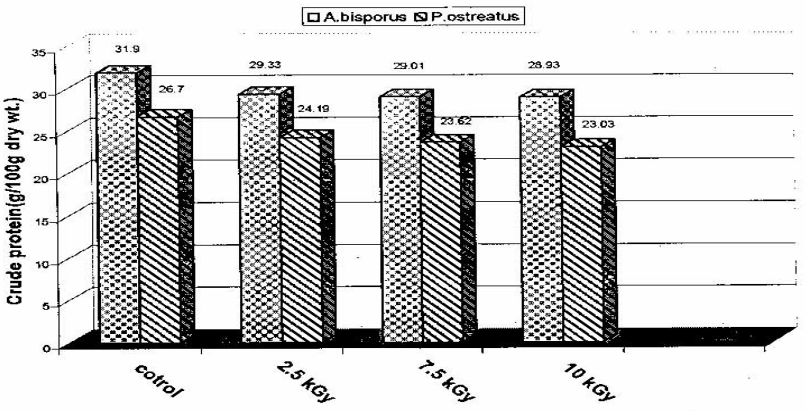

Fig. (1): Effect of different irradiation doses on crude protein (CP).

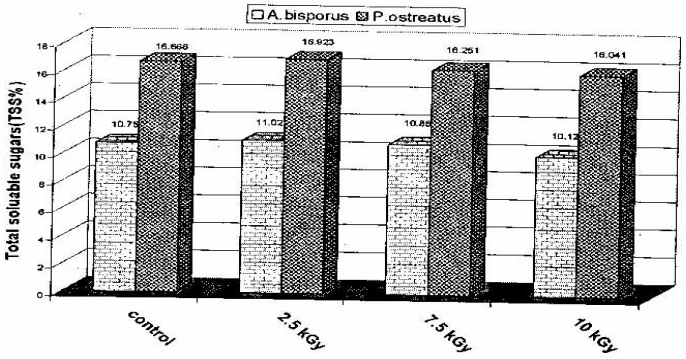

Fig. (2): Effect of different irradiation doses on total soluble sugars (TSS%).

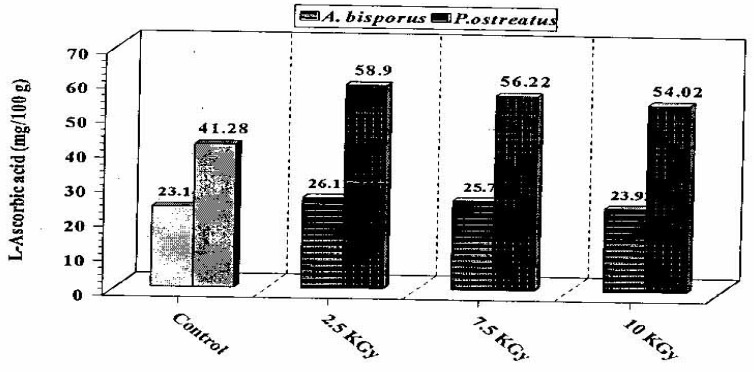

Fig. (3): Effect of different irradiation doses on L-ascorbic acid (mg/100g).

Diehl (1995) mentioned that vitamin losses from pure solutions are larger losses when the vitamin is in a food material and nutrient losses can be further minimized by irradiation food in a cold or frozen state.

Results given in Fig (4) and (5) showed that TPCs con centration (mg/g) and total flavonoids content (μ g/g) in tissues of *Agariucs Bisporus* were higher than those determined in tissues of *Pleurotus Ostreatus*. The irradiation process led to increases in TPCS. Wheras there are no remarkable changes in flavonoids due to the same treatments *Choi et al., (2006)* found that the free and bound polyphenolics of raw *shitake* were increased after heat treatment and this may be due to the disruption of the plant cell wall and thus bound polyphenolic and flavonoid componds may be released, also heat treatment could deactivate indogenous oxidative enzymes and this cause breakdown of the matrix so polyphenolic is increased *(Jeong et al., 2004)*.

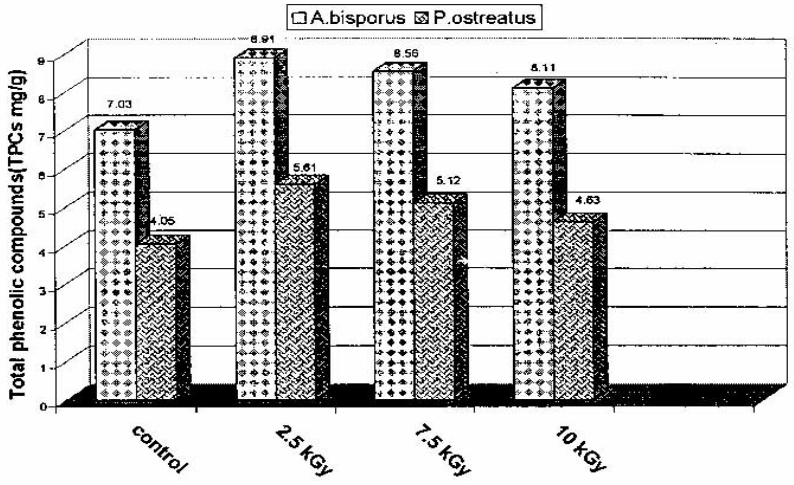

Fig. (4): Effect of different irradiation doses on Total Phenolic Compounds (TPCs).

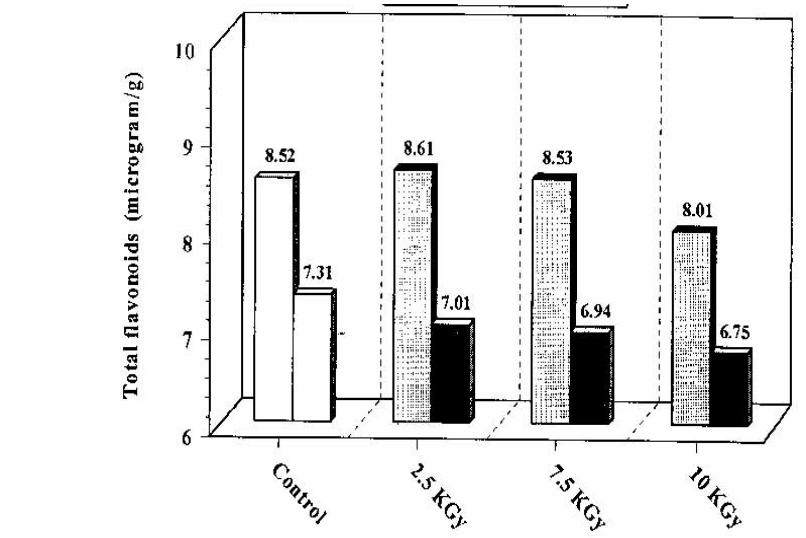

Fig. (5): Effect of different irradiation doses on Total flavonoids (TFs) µg/g.

Fig (6) showed significant increases in the total carotenoid contents of irradiated samples as compared to that of the control. Thses increase n caroteinod contents may be due to the degradation of pigments and changes in the conformation of the molecule.

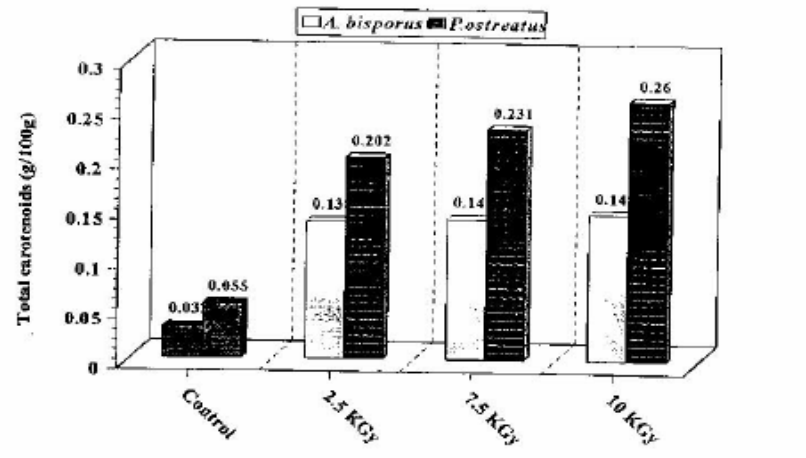

Fig. (6): Effect of different irradiation doses on Total Carotenoids (TCs) g/100g

Fig (7) and fig (8) showed the effect of irradiation on both plyphen aloxidase and tyrosinase activity, its cleared from fig (7) that the activity of polyphenoloxidase in *Agaricus Bisporus* was higher than that noticaed in *Pleurotus Ostreatus* and irradiated samples exhibited lower polyphenoloxidase activity compared to non-irradiated controls.

Fig (8) showed the effect of irradiation on activity of tyrosinase shich ws higher in *Aggaricus Bisporus* than that in *Pleurotus Osteatus* and the irradiated samples exhibited lowere tyrosinase activity compared to non-irradiated controls and that tyrosinase seemed to be the mjor phenoloxidase in the Agaricus straims, while *Oyster Mushrooms* had much lower levesls.

Khattak et al., (2008) mentiohed that increasing activity of enyme extract at higher doses of radiation due to some conformational changes.

Tyrosinase or polyphenoloxidase as present in plant tissues plays an important role in the quality of fruit and vegetable processing and during storage of the processed foods Prevention of browning in foods enzymatic or non enzymatic, has long been the concern of food scientists. *(khan and zakim 1995) , Espin et al., (1998).*

Tyrosinase is a copper – containing enzyme that in the presence of molecular oxygen, catalyzes two different reations : the hydroxylation of monopheonls to O-diphenols (monophenolase activity) and the oxidation of O-diphenols to O-guinones (diphenolase acititiy) which in turn non enzymatically polymerize to render brown, black, or red pigments (melanin's) *(Prota 1988 ., Martinez and Whitaker 1995).*

Table (1) indicated that peroxidase activity are low in the two types of mushroom examined, and the activity of the enzyme in *(Agaricus Bisporus)* was higher than that recorded in the other type of mushroom *(Pleurotus Ostreatus)*, and the activity of the enzyme in irradiated samples was lower than that the non- irradiated samples (control). And the phenoloxidase activity decreased during storage.

The obtained results are in good agreement with those found by *(Thomas et al., 1998, Beaulieu et al., 1992, and Ratcilffe et al., 1994).*

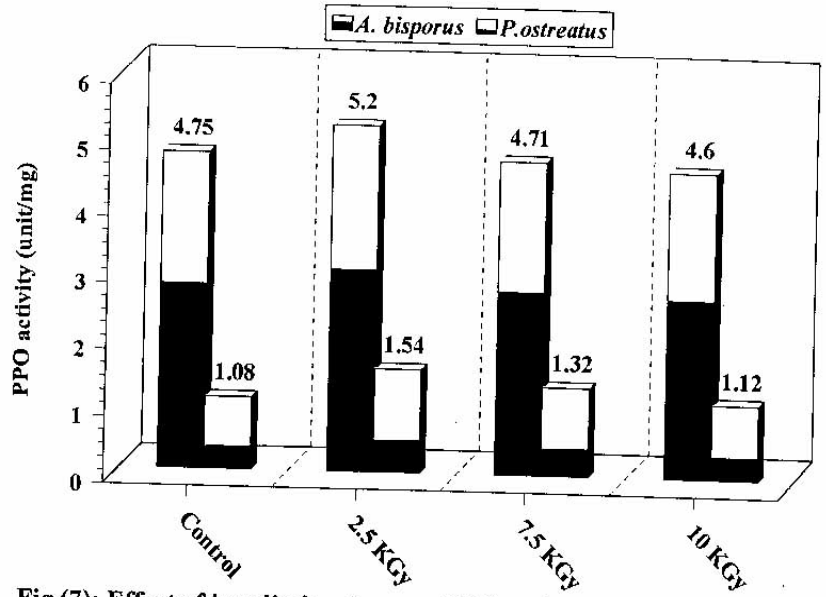

Fig (7): Effect of irradiation doses on PPO activity (unit/mg).

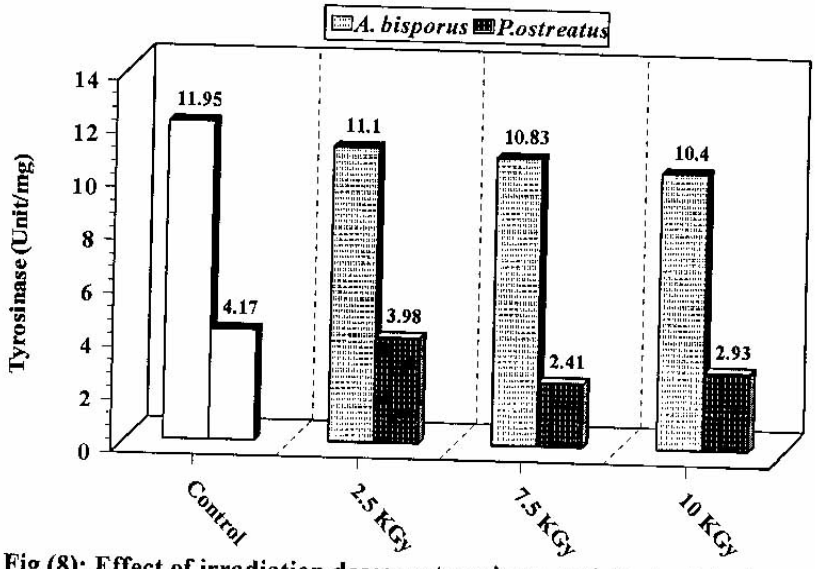

Fig (8): Effect of irradiation doses on tyrosinase activity (unit/mg).

Table (1) Effect of irradiation doses on peroxidase activity (unit/mg).

Treatments	Peroxidase activity (unit/mg).			
	control	2.5 KGy	7.5 KGy	10 KGy
Agaricus bisporus	18.25	17.99	17.82	17.73
Pleurotus ostreatus	12.53	11.98	11.40	10.98

Fig (9) showed that a remarkable change in the activities of phenyl- alanine ammonia – lyase (PAL) in tissues of *Agaricus Bisporus* and the effect of irradiation was at high dose on *Pleuratus Ostreatus*, resulted in higher decrease than that recorded in other sample.

A high level of proteases could play role in providing necessary amino acids that might connect complicted signaling pathways of differentiation. The role of cysteine proteases was uncertain, but one possible function may be in proteim turnover, with secretion of many hydrolytic enzymes and resulting development *(Shin and Choi 1998)* .

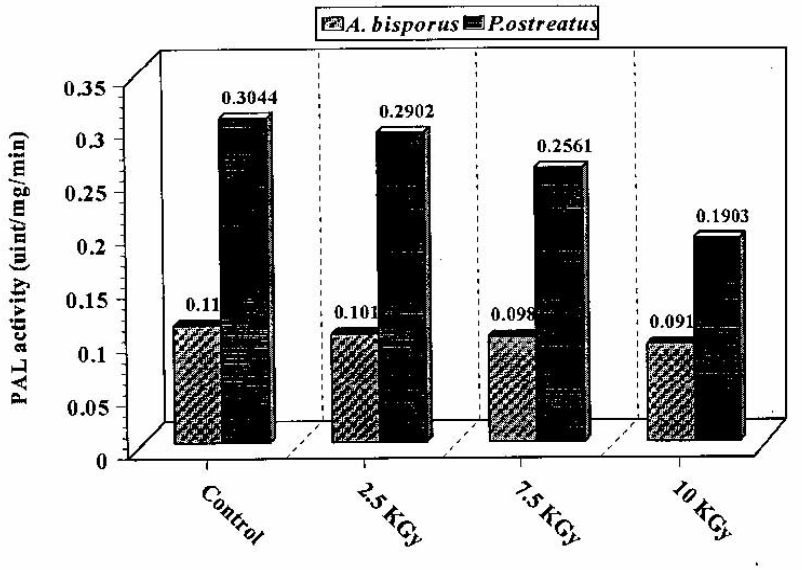

Fig (9): Effect of irradiation doses on PAL activity (unit /mg/min).

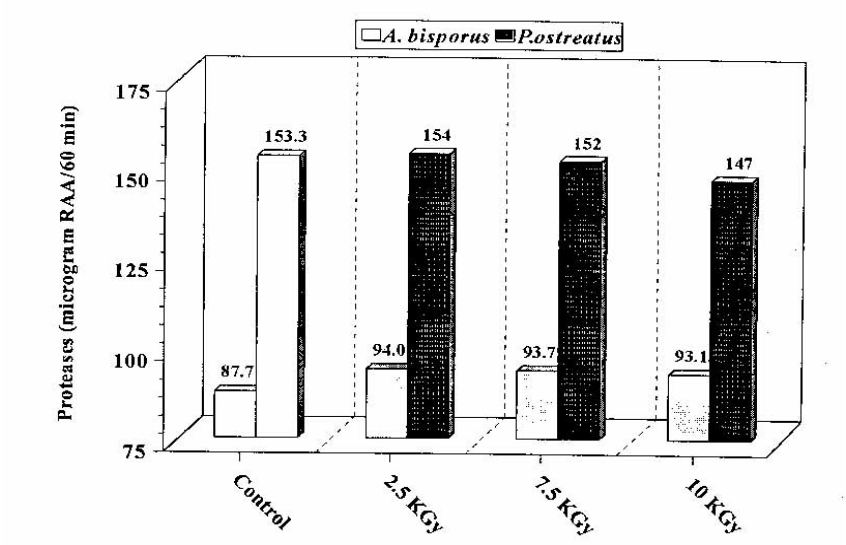

Fig (10): Effect of irradiation doses on proteases activity (µgRAA/60 min).

Fig (10) showed that the levels of proteases and the effect of different dose of irradiation and its cleared that the effect on *Agaricus Bisporus* and *Pleurotus Ostreatus* were limited except low irradiation dose (2.5 KGy) was varied than the others.

Several investigators *(kovacs and Vas., 1974, Skou et al, 1974, Thomas 1988 , and Beaulien et al., 1999)* reported that low-dose of gamma irradiation 2-5 KGy could be used to keep the quality of mushrooms and lowers the microorganism counts both initially and throughout the storage of mushrroms and increases the shelf life in addition such a dose causes oxidation of phenolic compounds present in vacuales, which could induce aslight brownish discoloration of mushrooms also *(Roy and Bahl 1984)* reported the necessary dose which decrease the browing effect is 2.5 KGy.

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تأثير التشعيع على المكونات الكيميائية لصنفين من عيش الغراب المأكولة

صلاح عبد العزيز ترك*- محمد عبد العزيز الشافعى*- محمد السيد رمضان*-

محمد ربيع أحمد جاد- حسن اسماعيل عبد الحكيم أحمد****

* قسم الكيمياء الزراعية -كلية الزراعة- جامعة المنيا – مصر

** معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعية –الجيزة

تهدف الدراسة إلى تقدير تأثير جرعات تشعيعية مختلفة من اشعة جاما بجرعات (2.5 ، 7.5 ، 10 كيلو جراى) على البروتين الخام والسكريات الكلية الذائبة وحمض الاسكوربيك والفينولات الكلية والكاروتينات الكلية وبعض الانزيمات فى عينيتين من عيش الغراب (المشروم) وهما *(Agaricus Bisporus)*، *(Aleurotus Ostreatus)*.